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Effect of an Acidified Milk on Diarrhoea and the Carrier State in Infants of Low Socio-Economic Stratum

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ABSTRACT. Brunser, O., Araya, M., Espinoza, J., Guesry, P. R., Secretin, M. C. and Pacheco, I. (Gastroenterology Unit, Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile and Nestec, Ltd., Vevey, Switzerland). Effect of an acidified milk on diarrhoea and the carrier state in infants of low socio-economic stratum. Acta Paediatr Scand 78: 259, 1989.

The effect on diarrhoeal disease of an acidified, modified powdered cow's milk infant formula (Pelargon®) was evaluated in 82 infants (Group I) for six months; 104 infants who received the same formula but non acidified, served as controls (Group II). Nutritional status remained satisfactory in both groups throughout the observation period. Some children rejected the taste of the acidified milk. The incidence of diarrhoea was lower in Group I ($p<0.001$). The proportion of days in which the children suffered from acute diarrhoea, and the duration of the episodes were also lower in the children given the acidified milk ($p<0.001$). The rate of detection of enteropathogens and the species identified were comparable in both groups. Carrier rates for bacterial enteropathogens fell over time in Group I while they rose in Group II ($p<0.001$). Carrier rates for enteric parasites were comparable to those expected in our setting for this age group. These results suggest that acidified milk exerts a protective effect against diarrhoeal disease. ***Key words: acidified milk, bacterial enteropathogens, carrier state, diarrhoeal disease.***

For many years, acidified milk has been considered to exert beneficial effects on gastrointestinal function (1). In 1923 Marriott & Davidson noticed that the pH of the gastric content was higher when undiluted cow's milk instead of human milk was fed to infants (2). Marriott postulated that neutralization of the buffering capacity of cow's milk would make it as digestible as human milk and would decrease the incidence of acute diarrhoea. In in vitro studies, acidified milk supports the growth of Lactobacilli and inhibits enteropathogens (3, 4). It is assumed that the same effect takes place in the intestinal lumen, and may help prevent diarrhoea. We undertook this prospective study to explore in a group of infants the effect of an acidified milk on the incidence, duration, symptomatology, aetiology and nutritional repercussions of diarrhoeal disease, as well as its influence on the carrier state.

PATIENTS AND METHODS

Infants whose medical care was provided at "Consultorio La Faena" and "Consultorio Peñalolén" in South Eastern Metropolitan Santiago were studied. Both centres are separated by approximately 15 blocks and serve a large, low income area. Two groups of infants were studied: all were under 12 months of age, they were 2500 g or more at birth, their weight/age was above 80% of the National Centre for Health Statistics 50th percentile (NCHS) (5), and they had been weaned spontaneously at least 15 days prior to admission to the protocol. None of them had received any antibiotics in the two weeks preceding the observation period and they were free of intercurrent diseases. The socio-economic stratum of the families and some characteristics of the houses were evaluated by means of standardized questionnaires (6, 7), an interview with the mother and observations at home. Group I, followed at "Consultorio Peñalolén", received the acidified milk and Group II, followed at "Consultorio La Faena", received the control milk. The acidified milk was a modified cow's milk formula (Pelargon®), containing 19 g protein, 33 g fat, 77 g carbohydrate and 2.3 g lactic acid/l. Its energy content was 2800 kJ (670 kcal/l) when re-

constituted. Acidification was achieved by addition of *Lactobacillus helveticus* and *Streptococcus thermophilus* (8). The formula contains enough vitamins and minerals to meet the daily requirements of growing infants. The control formula was identical except that it was not acidified.

A total of 146 infants received acidified milk. Of these, 72 dropped out during the first few days: 58 (80.5%) of these failures were because of the acid taste. It was therefore necessary to study 20 more infants; a total of 82 children completed the first month of observation because 12 of the newly enrolled infants rejected the acidified product. The control group comprised 132 children of whom 28 were lost to the study; only two (1.5% overall) because of taste. Each infant was given either the experimental or the control milk for 15 days prior to the observation period, to enable them to adapt to the new taste.

Between January and June 1986, each house was visited by a nurse twice weekly, who inquired about diarrhoea. If it was present, clinical information and faecal samples were collected: on the first day two rectal swabs were used for detection of bacterial enteropathogens and containers were provided for virological and parasitological specimens. An additional rectal swab on the following day completed the sampling for bacteriological assessment. Diarrhoea was defined as the passage of at least one liquid stool in 24 hours. To evaluate the carrier state for bacteria, a fixed number of 10 faecal samples were collected every month from among the asymptomatic children of each cohort using one swab. Asymptomatic children had to be free of diarrhoea for 15 days prior to and for seven days after the sampling. They should not have received any antibiotic treatments in the two preceding weeks. Medical care was provided at the Health Centres by members of our research team. Weight and height were measured monthly and acceptability of milks every 2 months.

Enteropathogens. Escherichia coli (enteropathogenic, toxigenic and invasive strains), Shigella, Campylobacter, Salmonella, rotavirus and enteroparasites were investigated by means of established techniques (9, 10).

This protocol was approved by the Committee on Ethics and Investigations on Human Subjects of the Institute of Nutrition and Food Technology (INTA), University of Chile.

RESULTS

All families belonged to the low socio-economic stratum of Chilean society. Both groups were comparable for housing, sanitary conditions and socio-economic characteristics. Children lost from Group I were also comparable for these characteristics and for age and education of both parents, number of members in the family and crowding.

The incidence of diarrhoea was lower in Group I ($z=5.536$; $p<0.001$) (11). Its distribution by age is shown in Table 1: the incidence of diarrhoea was higher for infants in Group II in all age intervals studied. The number of episodes per child detected during the study period

Table 1. *Incidence of episodes of acute diarrhoea in relation to age in children receiving acidified or control infant formulae, Santiago, Chile, 1985*

	Group I (acidified infant formula)				Group II (control infant formula)		
Age (months)	Children/ month	Episodes of diarrhoea	Incidence		Children/ month	Episodes of diarrhoea	Incidence
3–5	7	0	0	$\chi^2=7.123582$ $p<0.005$	18	3	16.7
6–8	81	8	9.9	$\chi^2=7.672687$ $p<0.005$	80	22	27.5
9–11	150	19	12.6	$\chi^2=15.0856$ $p<0.001$	159	41	25.8
12–15	165	12	7.3	$z=3.517$ $p^{***}<0.001$	329	71	21.6
Total	403	39	9.7		586	137	23.4

*** $p<0.001$ by z score (Ref. 11).

is shown in Table 2. Among the infants receiving the acidified milk, only 35% had diarrhoea during follow-up while 76% of those on the control milk had diarrhoea.

Taking into account the total number of days surveyed, children in Group I were ill with diarrhoea during 1.6% of the observation period while for those on the control milk this was 6.2% ($p<0.001$). The number of episodes per child for the observation period was 0.58 and 1.4 in Groups I and II, respectively.

A total of 28 enteropathogens (bacteria, virus or parasites) were detected in the 39 episodes (71.8%) in children from Group I and in 108 out of 137 episodes (78.8%) in Group II (p=NS). The enteropathogens identified during the episodes of diarrhoea are shown in Table 3. The rates of detection of each type of enteropathogen were also comparable. No differences could be established when the same rates were analyzed in relation to age.

The mean duration of the episodes was 6.8 days for Group I and 10.2 days for Group II ($p<0.001$). At the beginning of the episodes there were a few more cases of mild dehydration in Group I; however, the clinical course was uneventful in all infants as none of them required hospitalization. No episodes of prolonged diarrhoea (i.e. for 14 days or more) oc-

Table 2. *Distribution of episodes of acute diarrhoea per individual receiving acidified or control infant formulae, Santiago, Chile, 1986*

Group I (acidified infant formula)			Group II (control infant formula)		
No. of episodes of diarrhoea	No. of individuals affected	%	No. of episodes of diarrhoea	No. of individuals affected	%
0	63	65.0	0	25	23.8
1	30	30.9	1	39	37.1
2	3	3.1	2	31	29.5
3	1	1.0	3	6	5.7
4	0	0.0	4	2	1.9
5	0	0.0	5	2	1.9

Table 3. *Etiology of episodes of acute diarrhoea in children receiving acidified or control infant formulae*

EPEC = Enteropathogenic *E. coli*, ETEC = Enterotoxigenic *E. coli*, EIEC = Enteroinvasive *E. coli*

	Group I (acidified infant formula), n=39		Group II (control infant formula), n=137	
	n	%	n	%
Bacteria	21[NS]	75.0	74[NS]	68.5
EPEC	8	20.5	33	44.6
ETEC	5	12.8	4	5.4
EIEC	1	2.6	5	6.8
Salmonella	2	5.1	3	4.1
Shigella	1	2.6	15	20.3
Campylobacter	4	10.3	14	18.9
Parasites	5[NS]	17.9	24[NS]	22.2
G. lamblia	3	60.0	9	37.5
E. histolytica	2	40.0	15	62.5
Rotavirus	2[NS]	7.1	10[NS]	9.3
Total isolates	28	100.0	108	100.0

NS = not significant.

curred among children receiving acidified milk, while this occurred in eight infants (6%) of Group II.

None of the children in this study were undernourished according to anthropometric criteria. Comparison of the subgroups of children who suffered episodes of diarrhoea with those who did not, revealed no differences either.

Sixty-two faecal cultures were carried out in asymptomatic infants in Groups I and II, respectively. Of these, 24.2% were positive in Group I and 11.3% in Group II (p=NS) (Table 4). No differences were observed in relation to age. Comparing the positive results in the first two months of the study with those obtained in the last two months a decrease from 40% to 11.5% was observed in Group I while in Group II, this increased from 10.4% to 22.9% (χ^2=7.8150; p<0.001). Because the results in the experimental group contradicted those from previous studies performed in the same geographical area in this age group (12), we carried out a contemporary evaluation of parasitological infection in the same asymptomatic children who were completing the observation period. Twenty-five percent of the children in Group I had positive results compared to 16.3% in Group II (p=NS) (Table 4).

DISCUSSION

The milks were obviously different in taste and to avoid that the mothers discuss the characteristics of the products, this study was carried out at two different Health Centres. For this reason the groups were selected and proved to be comparable with respect to socioeconomic stratum, age and schooling of the parents, housing, availability of water and sewerage, and nutritional status of the infants (12, 13). These characteristics, including maternal attitudes towards the research team, were also comparable in those infants who dropped out early in the study.

Incidence of diarrhoea and its distribution by age in this study were significantly lower in children given acidified milk.

These infants were ill with diarrhoea less than one fourth of the time of those receiving the control milk (p<0.001). There is also a difference between the groups when results are expressed as the number of episodes per child during the study period. We also evaluated the proportion of children who became ill during the study period. In different surveys, some of them carried out in the same area where this study was implemented, we have observed that

Table 4. *Bacteria and parasites identified in asymptomatic children receiving acidified or control infant formulae*

	Group I: Acidified infant formula	Group II: Control infant formula
Bacteria	n=62	n=62
Enteropathogenic E. coli (EPEC)	5	1
Enterotoxigenic E. coli (ETEC)	3	1
Enteroinvasive E. coli (ETEC)	0	1
Campylobacter jejuni/coli	7	4
	15^{NS} (24.2%)	7^{NS} (11.3%)
Parasites	n=52	n=49
Giardia lamblia	10	5
Entamoeba histolytica	3	2
Ascaris lumbricoides	–	1
	13^{NS} (25%)	8^{NS} (16.3%)

NS = not significant.

40 to 74% of the children did not develop diarrhoea (13, 14, 15). In this study, 23% of the infants receiving the non-acidified formula remained free of diarrhoea. By contrast, 65% of the children given acidified milk did not have diarrhoea. The protective effect of the acidified milk seems to be further supported by the finding that while many infants given non-acidified milk had four or five episodes of diarrhoea, most infants given the acidified milk who developed diarrhoea had only one episode. The aetiologic agents detected during the episodes of diarrhoea did not differ in the two groups and the rates and the age distribution of these detections were comparable to those reported for comparable age groups in the same area (13–15). The clinical features of acute diarrhoea were similar in both groups. However, episodes were shorter in children on the acidified milk, and none of them lasted for more than 15 days.

One of the main problems in this investigation was the high proportion of children who refused the acidified milk. For this reason, changes in weight/age during the study period were evaluated. The fact that there was a normal weight increase suggests that the infants ingested enough of the milks in both groups.

An interesting finding in Group I was the decrease of carrier rates for bacterial enteropathogens, while asymptomatic parasitic infection was found at levels expected for this age group in our milieu (16). Although we have found that carrier rates may vary considerably from one place or one year to another, we had not previously detected decreases in this age group.

The results of this study suggest a protective effect of acidified milk. It remains to be determined why acidified milk prevents bacterial enteropathogens from colonizing the gut.

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